Predicted Outcome of Competition Among Ruminal Cellulolytic Bacteria for Soluble Products of Cellulose Digestion

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Introduction

Cellulose is the major component of forages and its digestion and subsequent fermentation by ruminal microbes provides much of the energy for forage-fed ruminants. Ruminal degradation of cellulose is mediated primarily by cell-associated enzymes produced by a few predominant bacterial species. The primary products of cellulose depolymerization are the soluble β-linked glucosyl oligomers (cellodextrins) cellobiose (G2), cellotriose (G3), cellotetraose (G4), cellopentaose (G5), and cellohexaose (G6). Of these, only cellobiose is commercially available in quantities needed for growth studies. Previously, we described the isolation of these cellodextrins in quantities sufficient for growth studies and determined the maximum growth rates (μ_{max}) and substrate concentrations permitting half-maximal growth ($S_{0.5\mu max}$) for two predominant cellulolytic species, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*. This report summarizes data for the third major cellulolytic species, *R. albus*, and uses these growth parameters to evaluate the probable outcome of competition for each substrate by the three cellulolytic species.

Methods

Mixed cellodextrins were prepared by hydrolysis of Sigmacell 20 microcrystalline cellulose with fuming HCl. Individual cellodextrins (G2-G6) were separated by charcoal/Celite/stearic acid column chromatography using a stepwise ethanol gradient. Identities of individual cellodextrins were determined by analytical HPLC using a Bio-Rad 42A Carbohydrate column eluted with H_2O. Growth experiments were conducted with glucose (G1) and each individual cellodextrin at 39°C in Balch-type anaerobic tubes containing a modified Dehority medium supplemented with various concentrations of each cellodextrin. Growth was monitored turbidimetrically at 600 nm, and growth rates determined as the linear portion of the regression line of ln (A_{600}) versus time. Maximum growth rates and substrate concentrations permitting growth at half-maximal rate were determined from Monod plots ($1/\mu$ vs. $1/S$). Two to four separate experiments were conducted for each cellodextrin/culture combination, with paired tubes for each cellodextrin concentration within each experiment.

Results

R. albus 7 appears to cleave cellodextrins of three or more glucosyl units prior to uptake, as has been noted for *R. flavefaciens* FD-1 and *F. succinogenes* S85. The Monod kinetic parameters for *R. albus* 7 (Table 1) indicate that this strain has μ_{max} values on most cellodextrins somewhat similar to those of the other two cellulolytic strains. While the values of $S_{0.5\mu max}$ are somewhat higher than for the other two strains for G1 and G2, there is a dramatic reduction in these values with increasing chain length.

By fitting the values of μ_{max} and $S_{0.5\mu max}$ to the Monod equation

$$\mu = \mu_{max} (S / S + S_{0.5\mu max}),$$

the growth rate of all three cellulolytic species could be predicted for each soluble sugar substrate (Fig. 1). The data indicate that *R. flavefaciens* FD-1 displays an ability to grow on each cellodextrin more rapidly than the other two species and that *F. succinogenes* S85 can generally grow more rapidly on glucose (a substrate not utilized by FD-1) and on most cellodextrins than can *R. albus* 7.

Table 1. Table 1. Monod kinetic parameters for *R. albus* 7 grown on glucose and cellodextrins.

Substrate	μ_{max} (h^{-1})	$S_{0.5\mu max}$ (mM)
G1	0.58[a]	4.16[a]
G2	0.48[a]	1.21[b]
G3	0.61[a]	0.41[c]
G4	0.53[a]	0.54[c]
G5	0.46[a]	0.20[d]

[a,b,c,d]Values within the same column having different superscripts differ significantly *(P < .05)*.

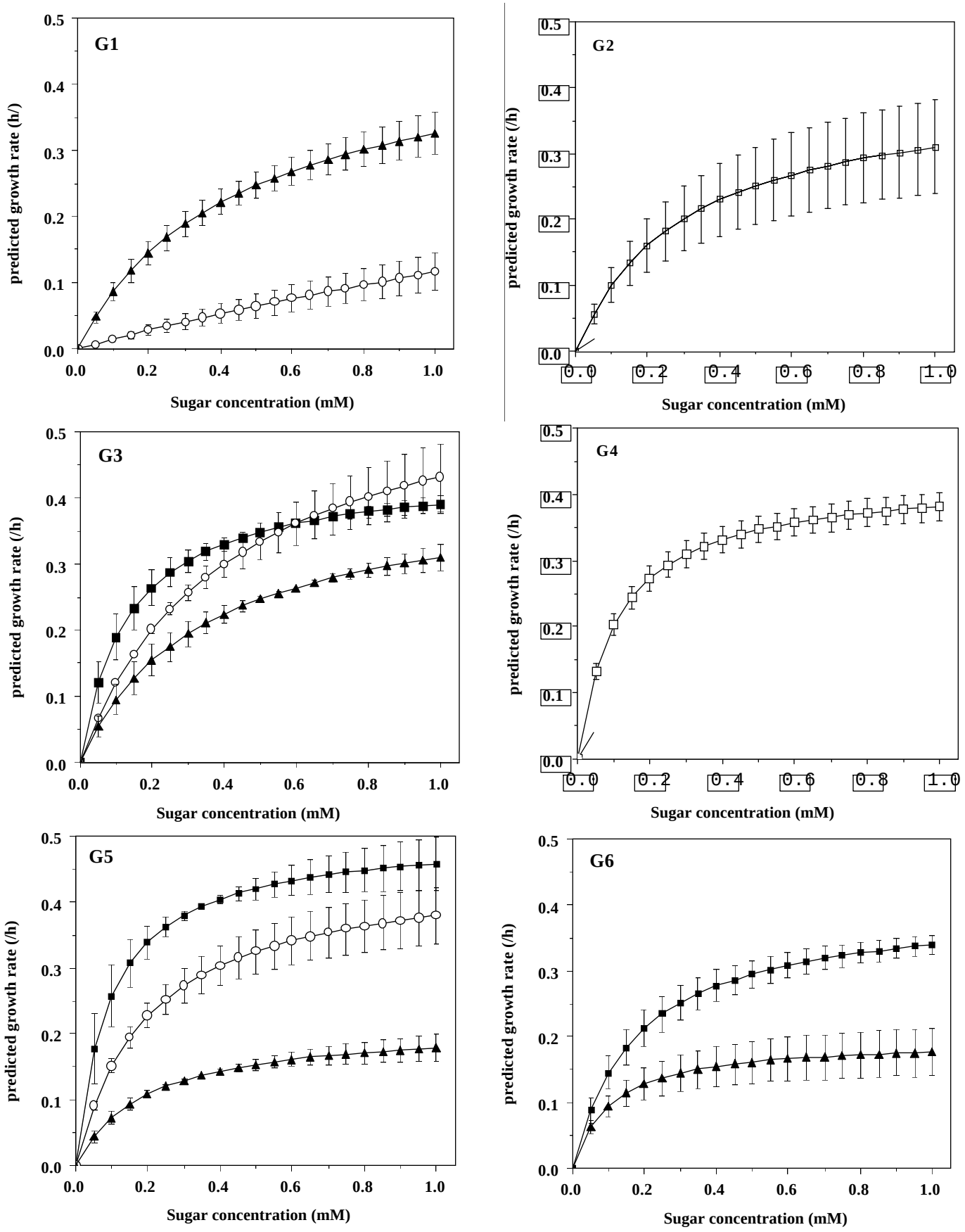

Figure 1. Predicted growth rates of three predominant ruminal cellulolytic bacteria, calculated from the Monod equation and the experimentally-determined values of μ_{max} and $S_{0.5\mu max}$. Error bars indicate 90% confidence intervals. ■, R. flavefaciens FD-1; ○, R. albus 7, ♦, *F. succinogenes* S85

Conclusions

The data suggest that *R. flavefaciens* FD-1 should outcompete *F. succinogenes* S85 and *R. albus* 7 for all cellodextrins regardless of their concentration if their interactions were based on pure and simple competition for soluble cellodextrins. While *F. succinogenes* should theoretically outcompete *R. albus* 7 for glucose, the low growth rate and poor affinity for this substrate relative to other glucose utilizing ruminal bacteria make glucose utilization by ruminal cellulolytic bacteria in the rumen environment unlikely.